REMARKS

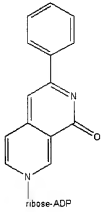
The amendment to claim 18 is supported by claim 19. Claims 19 and 23 have been cancelled. New claim 54 is supported by the specification at page 10, lines 8-12). New claim 55 is supported by the specification at page 7, lines 8-11.

No new matter has been added. Claims 18-39 and 54-55 are present in the application.

Request for Reconsideration

Applicants would like to thank Examiners L.D. Bland and D.M. Sullivan for the courteous interview held with Applicants' representatives on October 24, 2008. During the interview, the references cited in the Office Action of August 7, 2008 were discussed. Amending claim 18 to recite compound **1** was also suggested.

The present invention makes use of the discovery that treating a reaction mixture containing NAD+ with acetophenone in base, followed by heating during incubation with formic acid, yields compound **1**:



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Compound **1** is characterized by a strong fluorescence emission at 444 nm (page 6, lines 25-30). This allows for a fluorescent assay of NAD+ solutions as low as 10 pM, and the fluorescence is linear over the range of 1 nM – 100 μM (p. 7, lines 5-8). Importantly, nicotinamide does not react under the assay conditions, and NAD+ and nicotinamide have no significant intrinsic fluorescence emission at 444 nm (p. 7, lines 8-11).

Rejections – 35 U.S.C. § 102

The rejection of claim 18 under 35 U.S.C. § 102(b) as being anticipated by Weetall (U.S. Pat. No. 4,166,765) is respectfully traversed. The reference neither discloses nor reasonably suggests the converting of NAD+ to a fluorescent compound of formula **1**.

Weetall discloses a method for detecting enzymatic activity of 1,2-propandiol dehydrogenase, indicative of bacteria of the genus *Neisseria*, by monitoring changes in NADH concentration, observed fluorometrically at 460 nm, or as an increase in molar absorbance at 340 nm (column 2, lines 11-25). The author is silent as regards the conversion of NAD+ into fluorescent compound **1**.

As amended, claim 18 recites converting any remaining NAD+ to compound **1**. Weetall only discloses the conversion of NAD+ to NADH, not compound **1**. The reference is therefore not anticipatory of the claimed invention, and withdrawal of this ground of rejection is respectfully requested.

Rejections – 35 U.S.C. § 103

The rejection of claims 18-24 under 35 U.S.C. § 103(a) as being unpatentable over Clark et al. (Analytical Biochemistry 68, 54-61 (1975)) in view of Osawa et al. (Journal of Clinical Microbiology, April 1997, p. 951-953), as evidenced by Nakamura et al. (Analytical Chemistry, Vol. 50, No. 14, December 1978, p. 2047-2051), and further in view of Pieper et al. (PNAS, February 15, 2000, vol. 97, No. 4, p. 1845-1850), is respectfully traversed.

The fact that references can be combined or modified may not be sufficient to establish prima facie obviousness: "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art." M.P.E.P. § 2143.01 (III), citing KSR International Co. v. Teleflex Inc., 550 U.S. ___, ___, 82 USPQ2d 1385, 1396 (2007).

Clark et al. discloses a fluorimetric assay for N^1-methylnicotinamide (abstract). N^1-methylnicotinamide is converted to a fluorescent derivative by addition of acetophenone and KOH in 80% ethanol (page 61, second paragraph), followed by

addition of 99% formic acid (page 56, second full paragraph). No heating following the addition of the formic acid is disclosed.

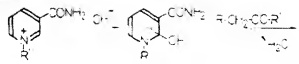
The fluorescence was measured at an emission wavelength of 430 nm. In KOH 0.5 N and 80% (V/V) ethanol, the nanomolar relative fluorescence of N^1-methylnicotinamide was observed to be about 4. The nanomolar relative fluorescence obtained under the same conditions for NAD+ was approximately 0.15 (page 56, last paragraph to page 57, first paragraph). The authors concluded that "NAD+ [...] yield[s] derivatives with a molar fluorescence about 1/25 that of methylnicotinamide" (page 61, second paragraph). Such derivatives were not further characterized; in particular, no fluorescence emission at 444 nm by NAD+ derivatives is reported.

Nakamura et al. discloses a spectrofluorometric method for the determination of α-methylene carbonyl compounds by using N^1-methylnicotinamide chloride (NMN) (abstract). An alkaline mixture was formed by adding NaOH 6M and NMN to an aqueous sample. After a period of time, the solution was acidified with formic acid 18.66 M. The resulting acidic solution was heated to 92 °C for 3 minutes and then cooled (page 2047, second column, fifth paragraph). Only α-methylene carbonyl compounds having a general formula $R\text{-}CH_2\text{-}CO\text{-}R'$ gave intense fluorescence (page 2050, first column: Table I and last paragraph). Additional heating was required to obtain maximal fluorescence (page 2050, second column, first full paragraph).

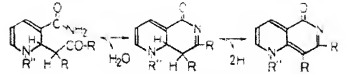
Nakamura et al. never carries out a reaction with NAD+ as a reagent. Rather, Nakamura et al. cites a number of references, including Clark et al., as disclosing a procedure involving the reaction of N^1-alkylpyridinium compounds with ketones in alkaline media, followed by heating with excess acids to produce fluorophores (page 2047, first column, last paragraph). As noted above, however, Clark et al. discloses no such heating.[1]

Furthermore, Nakamura et al. proposes the steps of Scheme I as leading to fluorophore products where the two ring nitrogen atoms are positioned relative to each other as in a 1,6-naphthyridine molecule:

Scheme I



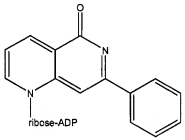
α-CARBINOL



α-ADDUCT CYCLIZED α-ADDUCT (NONFLUORESCENT) FLUOROPHORE

(Nakamura et al., page 2047, first column).

Therefore, according to Nakamura et al., treating NAD+ with acetophenone in base, followed by heating in an acidic medium, would be expected to produce a compound of Formula **1'**:



Formula **1'**

In the claimed invention, the NAD+ is converted to compound **1**, where the two ring nitrogen atoms are positioned relative to each other as in a 2,7-naphthyridine molecule. This is achieved by treating a reaction mixture containing NAD+ with acetophenone in base, followed by heating during incubation with acid. Clark et al. does not disclose or suggest heating during incubation with acid. Furthermore, the steps of Scheme I of Nakamura et al. cannot lead to compound **1**, nor does Nakamura et al. carry out NAD+ assays involving treating NAD+ with acetophenone in base, followed by heating in an acidic medium. Because of these dissimilarities, Clark et al.

and Nakamura et al. provide no basis on which one could predict the formation of compound **1**.

Osawa et al. discloses a method for identifying cholera-enterotoxin (CT)-producing *Vibrio cholerae* serogroups (abstract). The method includes measuring the concentration of NAD+ by means of a color-amplifying solution (paragraph bridging pages 951 and 952). The authors are entirely silent as regards fluorescent derivatives of NAD+. Pieper et al. discloses monitoring PARP activity through conversion of radioactively labeled [^{32}P]NAD+ to labeled PAR (page 1845, second column, last paragraph; page 1846, second column, second paragraph). No fluorescence-based methods are used or suggested.

As claimed, the method of the invention converts NAD+ to compound **1**. Furthermore, the compounds of the cited references would not have led one of ordinary skill in the art to predict the formation of compound **1**. Accordingly, the claimed invention is not obvious over the applied references, and withdrawal of this ground of rejection is respectfully requested.

Rejections – 35 U.S.C. § 112

The rejection of claims 18 - 24 under 35 USC § 112, first paragraph, is respectfully traversed. An enormous number of enzymes utilizing NAD+ are known in the art, and there is no need for Applicants to describe them. In addition, the present application describes how to assay solutions containing NAD+.

The description need only describe in detail that which is new or not conventional. M.P.E.P. § 2163(3)(a) (citing Hybritech v. Monoclonal Antibodies, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986) and Fonar Corp. v. General Electric Co., 107 F.3d 1543, 1549, 41 USPQ2d 1801, 1805 (Fed. Cir. 1997)). Applicants' invention is not enzymes that utilize NAD+, rather the claimed invention provides methods for measuring the activity of such enzymes.

Enzymes utilizing NAD+ were well established in the art as of the filing date for the instant application. NAD+ is found in all living cells and is a coenzyme for a great number of enzymes other than PARP. Applicants highlight examples of such enzymes in their specification, namely dehydrogenases such as aldehyde dehydrogenase (page

10, lines 8-12) and long-chain 3-hydroxyacyl-CoA dehydrogenase (page 10, lines 23-26).

Applicants' invention is not enzymes that utilize NAD+, rather the claimed invention provides methods for measuring the activity of these well known and studied enzymes. Since enzymes that utilize NAD+ are old and well known, there is no need for Applicants to describe them. Withdrawal of the claim rejections under 35 U.S.C. § 112, first paragraph, is respectfully requested.

The rejection of claims 18-24 under 35 U.S.C. § 112, second paragraph is respectfully traversed. The elements "an NAD+ utilizing enzyme" and "a substrate for the enzyme" are recitations of properties of the claimed methods. Furthermore, as presently amended, independent claim 18 recites structure **1** as the "fluorescent compound". One of ordinary skill in the art can determine whether a method reads on the respective claims by applying the test recited in the claims, and measuring the amount of fluorescence of the fluorescent compound **1**. Accordingly, the claims are consistent with the requirements of 35 U.S.C. § 112, second paragraph. Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants respectfully submit that the application is now in condition for allowance. Early notice of such action is earnestly solicited. Should the Examiner feel a discussion would expedite the prosecution of this application, the Examiner is kindly invited to contact the undersigned at (312) 876-1400.

Respectfully submitted,

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